MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, June 2004, p. 362–372 Vol. 68, No. 2
1092-2172/04/$08.00+0 DOI: 10.1128/MMBR.68.2.362–372.2004

Pathogenesis of Human Papillomaviruses in Differentiating Epithelia

Michelle S. Longworth and Laimonis A. Laimins*

Department of Microbiology-Immunology, The Feinberg Medical School, Northwestern University, Chicago, Illinois 60611

HUMAN PAPILLOMAVIRUSES AND CERVICAL CANCER

Human papillomaviruses (HPVs) are small, double-stranded DNA viruses that infect cutaneous and mucosal epithelial tissues of the anogenital tract, the hands, or the feet. A subset of HPV types are the causative agents of cervical cancer, since 99% of tumors are positive for HPV DNA (150). To date, over 100 different viral types have been identified, and about one-third of these infect epithelial cells in the genital tract. The viral types that infect the genital tract fall into two categories: high risk and low risk. The high-risk types are associated with the development of anogenital cancers including those of the cervix, while infections by the low-risk HPVs induce only benign genital warts. The high-risk types include HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45, while the low-risk types are HPV-6 and HPV-11. HPVs that infect the genital tract are sexually transmitted, and it is estimated that about two-thirds of individuals who have sexual relations with an infected partner will themselves become infected. However, the majority of infections are subclinical (137). Infection by high-risk HPVs is not limited to the genital tract, since approximately 20% of cancers of the oropharynx contain DNA from these HPV types (61).

Infection of the genital tract by HPVs can initially result in low-grade lesions termed dysplasias or cervical intraepithelial neoplasia grade I. These lesions exhibit only mildly altered patterns of differentiation, and many of them are cleared by the immune system in less than a year (62, 71). The mechanisms by which the cellular immune response clears HPV infections are still not clearly understood. Some of these lesions, however, are not cleared by the immune system and can persist for periods as long as several decades. Persistence of infection by high-risk HPV types is the greatest risk factor for development of genital malignancies such as squamous cell carcinoma or, less commonly, adenocarcinoma of the cervix (161). Cervical cancer is the second most prevalent cancer worldwide and is the fifth leading cause of cancer deaths in women (120, 124). Approximately 470,000 new cases of cervical cancer are diagnosed yearly, with the mean age for the development of malignancy being 52 years (8, 124). Risk factors for tumor development include persistent infection with high-risk viral types, a large number of lifetime sexual partners, coinfection with human immunodeficiency virus, immunosuppression, and cigarette smoking (81). Most cases of cervical cancer are found outside of the United States and Western Europe. In the United States, the number of cases of cervical cancer has declined by over 80% in the last 50 years due to the implementation of the Pap smear as a diagnostic (137). While the number of cases has significantly decreased, approximately 10,000 women are diagnosed with cervical cancer and 5,000 die of this disease annually (120).

HUMAN PAPILLOMAVIRUS LIFE CYCLE

HPVs are nonenveloped viruses with icosahedral capsids that replicate their genomes within the nuclei of infected host cells. The double-stranded, circular DNA genomes of all HPVs are approximately 8 kb in size. In virions, the HPV DNA is found associated with cellular histones to form chromatin-like complexes (63). The viral genomes carry on average eight major open reading frames (ORFs), and these are expressed from polycistronic mRNAs transcribed from a single DNA strand (Fig. 1). In the high-risk HPV types, transcripts are initiated at two major viral promoters, one of which initiates upstream of the E6 open reading frame, encodes early viral proteins, and is expressed prior to productive replication. In HPV-16 and HPV-31 this promoter is referred to as p97, while in HPV-18 it is referred to as p105. Coincident with the induction of productive replication, the late promoter is activated, which directs expression from a series of heterogeneous start sites clustered around nucleotide 742 (p742) in HPV-31 (68). Similar promoters have been identified in HPV-16 and HPV-

*Corresponding author. Mailing address: Department of Microbiology-Immunology, The Fineberg Medical School, Northwestern University, 303 E. Chicago Ave., Chicago IL 60611. Phone: (312) 503-0648. Fax: (312) 503-0649. E-mail: l-laimins@northwestern.edu.

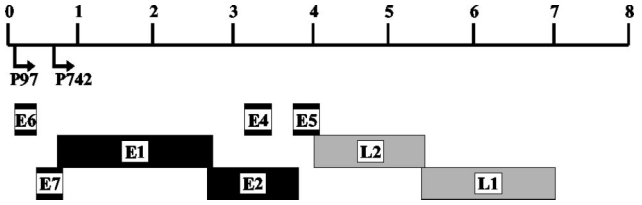

FIG. 1. Genomic organization of high-risk HPV-31. Early ORFs are indicated in black, while capsid genes are shown in gray. Early and late promoters are designated by arrows.

18 (46, 53). Several additional minor promoters have been identified that also play important roles during the viral life cycle (117).

The life cycle of HPV is linked to the differentiation program of the infected host cell, the keratinocyte, with production of mature virion particles restricted to differentiated suprabasal cells. Infection by papillomaviruses is thought to occur through microwounds of the epithelium that expose cells in the basal layer to viral entry. The receptor for entry of the virus into cells is currently unknown; however, heparin sulfate mediates the initial attachment of virions to cells (Fig. 2) (76). Cells in the basal layer consist of stem cells and transit-amplifying cells that are continuously dividing and provide a reservoir of cells for the suprabasal regions (68). HPV infection of these cells leads to the activation of a cascade of viral gene expression that results in the production of approximately 20 to 100 extrachromosomal copies of viral DNA per cell. This average copy number is stably maintained in undifferentiated basal cells throughout the course of the infection. Among the first viral proteins to be expressed are the replication factors, E1 and E2. These proteins form a complex that binds to sequences at the viral origin of replication and acts to recruit cellular polymerases and accessory proteins to mediate replication (18, 44, 107). The E1 protein also exhibits helicase activity, allowing for the separation of viral DNA strands ahead of the replication complex (65). E2 is a site-specific DNA binding protein that helps to recruit E1 to the origin but also plays a role in regulating viral transcription from the early promoter (21). Binding sites for E2 are located adjacent to sites for cellular transcription factors that activate the early promoter (142). At low levels, E2 binds its recognition sequences and activates the early promoter, while at high concentrations, it represses by blocking the binding of cellular transcription factors (139). Since the E1 and E2 replication factors are also expressed from the early promoter, the ability of E2 to activate and repress expression contributes to the control of viral copy number in undifferentiated cells.

The E6 and E7 proteins of the high-risk HPV types act as viral oncoproteins, but no such functions are associated with the corresponding proteins from the low-risk types. High-risk E6 binds the p53 tumor suppressor protein as part of a trimeric complex with the cellular ubiquitin ligase, E6AP, leading to the rapid turnover of p53 (131, 155). E7 binds to the retinoblastoma (Rb) family of tumor suppressors, as well as other proteins involved in cell cycle regulation (37, 110). As HPV infected basal cells divide, viral genomes are partitioned into daughter cells, one of which detaches from the basal layer, migrates toward the stratum granulosum, and undergoes differentiation (Fig. 2). In normal uninfected epithelia, cells exit the cell cycle as they leave the basal layer, and this often results in the loss of nuclei in suprabasal cells. As infected cells leave the basal layer, they remain active in the cell cycle due to action of the E7 protein (15). Cells reenter the S phase in highly differentiated cells and activate the expression of cellular replication factors required for viral replication. The presence of E7 leads to a characteristic retention of nuclei throughout all layers of infected epithelia. Not only are the viral oncoproteins necessary for cell immortalization and retention of cell cycle capability on differentiation, but E6 and E7 have also been shown to be necessary for the maintenance of extrachromo-

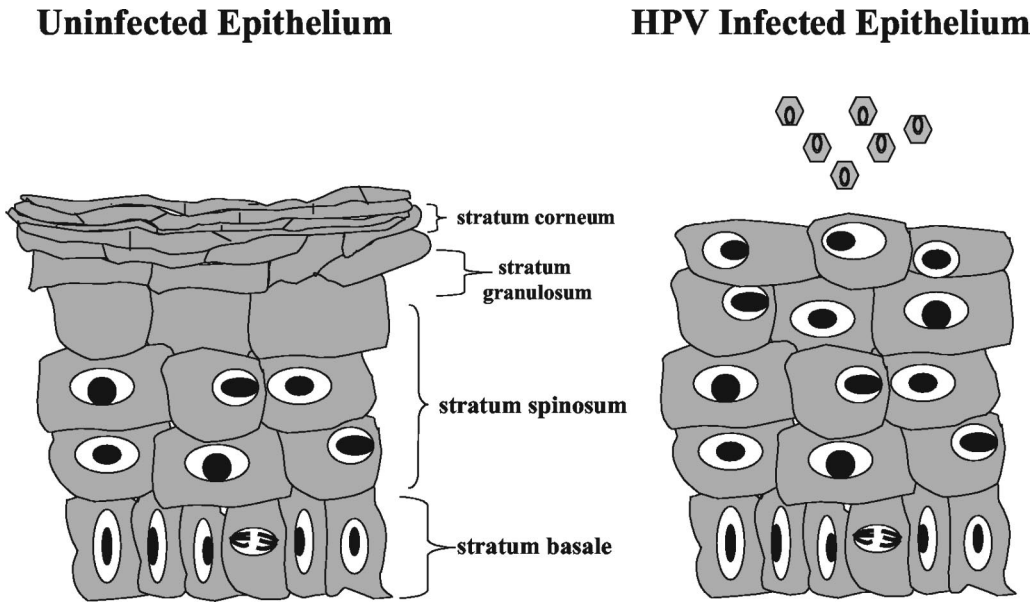

FIG. 2. Cartoon of uninfected (left) and HPV-infected (right) epithelia showing various differentiated layers and virion production.

somal forms of HPV in undifferentiated basal cells (145). The mechanism by which this occurs is not clear, although it probably likely involves abrogation of checkpoints that block the long-term retention of the extrachromosomal DNAs. The functions of the E4 and E5 proteins are not yet fully understood; however, they both have been proposed to be involved in regulation of late viral functions (40). The L1 and L2 proteins are assembled late and spontaneously form icosahedral capsids. Following virion assembly, mature viruses are released from the uppermost layers of the epithelium (Fig. 2) (68).

It is still not clear how the differentiation program of the host cell is able to activate the productive life cycle of HPV. The most likely mechanism centers on the activation of expression of the late viral promoter, resulting in high-level expression of transcripts encoding the viral replication proteins, E1 and E2, along with the late genes. Unlike the early promoter, the late promoter is not negatively regulated by E2 protein, and high levels of expression occur upon differentiation, leading to amplification of viral DNA (139). This increase in template numbers results in a further increase in expression of the replication proteins. It is possible that cellular or other viral factors are upregulated on differentiation and that these factors contribute to activation of late functions, but the identification of these proteins is only beginning. In low-grade infections, the high-risk HPV genomes are present as episomes, while during progression to high-grade lesions or carcinomas, the genome often is found integrated into host sequences. This integration usually occurs within the E2 ORF and results in loss of E2 repressive action leading to higher levels of E6 and E7 expression (2, 4, 5, 72, 133, 134). In the following sections, we describe in detail the present state of knowledge about how the viral factors act during the productive life cycle as well as during progression to malignancy.

REGULATION OF HUMAN PAPILLOMAVIRUS GENE EXPRESSION IN DIFFERENTIATING EPITHELIA

Two major promoters direct the expression of HPV ORFs during the early and late phases of the viral life cycle. Most HPV mRNAs are polycistronic, and many carry three or more ORFs. Expression of various early genes is regulated, in part, by alternative splicing mechanisms. Translation of viral proteins is thought to occur through a leaky scanning mechanism rather than through the use of IRIS elements and provides an additional level of regulation. The major early promoter of high-risk HPVs directs transcription at sites upstream of the E6 ORF. The transcription factors that regulate the early promoter bind to sites located within a region upstream of E6 of about 1 kb that has been called either the long control region or the upstream regulator region (URR). In this review, we use the designation URR. Examination of URR regions of different HPV types has identified a number of binding sites shared among all types as well as some that are unique. Common binding sites include those for TFIID binding to canonical TATA boxes located approximately 30 bp upstream from the early start sites. Upstream of these sequences are Sp-1 and AP-1 binding sites, and these are found in all HPV types studied (27). Additional factor binding sites shared among many HPV types include those for NF-1, TEF-1, TEF-2, Oct-1, AP-2, KRF-1, and YY1, as well as glucocorticoid responsive elements (11, 12, 49, 70, 88, 115). Keratinocyte-specific enhancers have been identified in the URR regions, and these elements contribute to the tissue tropism of HPVs. It has been hypothesized that it is the combination of ubiquitous factors working in concert that determines the cell-type-specific expression (12).

Late viral transcription is activated on epithelial differentiation from start sites located within the E7 ORFs of the genital HPV types. The tight linkage of viral late-gene expression and epithelial differentiation suggests that differentiation-specific cellular factors control this process. Little information is available about the identity of these factors, but it has been shown that a rearrangement of chromatin occurs around the late promoter region on epithelial differentiation (27). With the availability of methods for genetic analysis of HPV functions during the viral life cycle, a detailed examination of the factors involved in differentiation-dependent activation of the late promoter is now possible.

FUNCTIONS OF THE E6 ONCOPROTEIN

The E6 proteins of both high- and low-risk types are approximately 150 amino acids in size and contain two zinc binding domains with the motif Cys-X-X-Cys. The high-risk E6 proteins are distributed to both the nucleus and the cytoplasm and have been reported to bind to over 12 different proteins (162). Expression of high-risk E6 alone leads to the transformation of NIH 3T3 cells as well as the immortalization of human mammary epithelial cells (79, 96). In contrast, efficient immortalization of human keratinocytes requires the expression of both E6 and E7 (58). Many of the first insights into the action of E6 have come by studying its interactions with p53. p53 is a well-characterized tumor suppressor that regulates the expression of proteins involved in cell cycle control, including the cyclin kinase inhibitor, p21 (84). On exposure to DNA damage, p53 becomes activated and induces high-level expression of p21, resulting in cell cycle arrest as well as apoptosis (84). One way in which infected cells act to prevent the spread of viruses to neighboring cells is through the activation of apoptotic pathways. Many viruses have evolved mechanisms to block the induction of apoptosis, but this can, in some cases, allow for the development of malignancies. To overcome the proapoptotic activities of p53 and allow for cell cycle progression, E6 binds to p53 in a ternary complex with a ubiquitin ligase called E6AP (66). Formation of this complex results in the ubiquitination of p53 and subsequent degradation by the 26S proteasome, leading to a reduction in the half-life of p53 from several hours to less than 20 min in keratinocytes (64, 67). E6 can also indirectly downregulate p53 activity through its association with p300/CBP, which is a coactivator of p53 (91, 160). Since p53 regulates both the G_1/S and G_2/M checkpoints of the cell cycle, its rapid turnover results in abrogation of these controls, leading to chromosomal duplications and centrosomal abnormalities (42, 78, 147). An alternate form of E6, called E6*, is generated as a result of splicing and has been shown to interact with both E6 and E6AP (123, 130). E6* may function by inhibiting the degradation of p53, but immortalization is mediated only by the full-length E6 protein (6).

Interestingly, the binding of E6 to E6AP also results in the self-mediated ubiquitination of E6AP (77). This raises the

possibility that E6 may regulate the levels of the natural cellular substrates of E6AP though degradation of E6AP. Some of the proteins targeted for degradation by E6AP include members of the Src family of tyrsosine kinases which interact with a variety of signaling networks (43). While it has not been shown whether E6 can directly regulate Src family kinases through E6AP, this represents an interesting area for future study.

Recent studies have identified p53-independent activities of E6 that are important for immortalization of human cells. E6 mutants of HPV-16 have been identified that are unable to degrade p53 but retain the ability to immortalize mammary epithelial cells Similarly, other E6 mutants retain the ability to degrade p53 but have lost the ability to immortalize (79, 96). These data suggest that interactions with proteins other than p53 may be essential for immortalization of cells. One such important interaction appears to be that of E6 with the PDZ family of proteins. PDZ proteins contain a conserved domain that associates with the PSD-95, Dlg, and ZO-1 proteins (hence the name PDZ). The PDZ domain is often found in proteins localized at areas of cell-cell contact, such as tight junctions in epithelial cells. The PDZ proteins probably act as molecular scaffolds to aid in signal transduction (20, 50). The binding of PDZ family members MUPP-1, hDLG, and hSCRIB to the extreme C terminus of high-risk E6 proteins results in the degradation of the PDZ protein (80, 92, 93, 111). The importance of this interaction has been confirmed in experiments with transgenic mice expressing E6 proteins that lack the PDZ binding domain. These mice retain the ability to inactivate p53 but do not develop the epidermal hyperplasias that are frequently seen in wild-type E6 transgenics (114). It is not clear which signaling pathways are impacted by E6 binding to PDZ proteins or which PDZ family members are most important for these phenotypes. The elucidation of these pathways is of great importance for our understanding of HPV pathogenesis.

Another major function of the high-risk E6 proteins that is important for immortalization is their ability to activate the expression of the catalytic subunit of telomerase, hTERT (82, 106, 112). Telomerase is a four-subunit enzyme that adds hexamer repeats to the telomeric ends of chromosomes. Telomerase activity is usually restricted to embryonic cells and is absent in somatic cells. The lack of telomerase activity results in a shortening of telomeres, with successive cell divisions eventually leading to senescence (94). In contrast, in most cancers, reactivation of hTERT expression occurs and leads to reconsitution of telomerase activity (94). E6 appears to activate hTERT transcription through the combined action of Myc and Sp-1 (54, 89, 116, 152, 156). Recent studies have shown that E6 can bind to bind both Myc and its cofactor Max, leading to transcriptional activation of the hTERT promoter (149). Analysis of E6 mutants that discriminate between the ability to degrade p53 and to activate hTERT demonstrated that the latter activity is most important for immortalization. However, immortalization of human foreskin keratinocytes (HFKs) also requires inactivation of the Rb pathway through either mutation of cellular genes or the presence of E7 (41, 79, 109). It appears that p53 degradation is most important for full transformation and that binding of PDZ proteins and activation of hTERT expression are necessary for immortalization.

The high-risk E6 proteins interact with a number of other cellular proteins, such as paxillin, p300/CBP, the putative calcium binding protein E6-BP, and the interferon regulatory factor IRF-3 (121, 128,160). While most of these interactions are specific for the high-risk forms of E6, several reports have documented cellular binding partners for low-risk E6 proteins, such as zyxin, GPS2, Bak, and McM7 (25, 26, 86, 87, 146). It has also been reported that both high- and low-risk E6 proteins can bind to p73, but this has not been seen by other investigators (99, 119). The physiological relevance of each of the interactions described above is not yet fully understood and will probably require an analysis of their roles in the productive life cycle.

Clearly, the primary role of E6 in the productive viral life cycle is not in inducing transformation or immortalization but is more likely to involve facilitation of some aspect of viral replication. Using a genetic system involving transfection of cloned HPV genomes into keratinocytes, it has been demonstrated that expression of functional E6 is required to maintain HPV-31 and HPV-11 genomes as stably replicating episomes (116a, 145). This may reflect a shared requirement for abrogation of the mechanisms that prevent the maintainance of extrachromosomal DNAs. Further elucidation of the roles of these proteins in the viral life cycle remains an active area for future studies.

THE E7 PROTEINS INTERACT WITH THE RETINOBLASTOMA PROTEINS AND HISTONE DEACETYLASES

The second HPV oncoprotein that is important for both immortalization and viral pathogenesis is E7. E7 proteins of both high- and low-risk types are found predominantly in the nucleus and are approximately 100 amino acids in size. Expression of E7 by itself is able to transform immortalized NIH 3T3 mouse cells and, at a low frequency, to immortalize the natural host cell, human keratinocytes (109, 126). However, the efficient immortalization of human keratinocytes requires the action of both the high-risk E6 and E7 proteins (109). Transgenic mice expressing E7 alone develop both low-grade lesions and high-grade cervical dysplasias that can progress to malignancy, while E6 transgenic mice develop only low-grade hyperproliferative lesions (126). Central to the action of the E7 proteins is their ability to associate with the Rb family of proteins (37). The binding of Rb is mediated through one of three conserved regions present in all high risk E7 proteins: CR1 at the N terminus; CR2, which contains an LXCXE motif that binds the Rb protein; and CR3, which contains two zinc finger-like motifs. The CR1 and CR2 domains of E7 have sequence homology to the adenoviral E1A CR1 and CR2 domains that also bind to Rb proteins (122).

The Rb family of "pocket" proteins includes Rb, p107, and p130, and these proteins are differentially expressed throughout the cell cycle (7, 17, 37). While Rb is constitutively expressed throughout all phases of the cell cycle, p107 is synthesized predominantly during the S phase and p130 predominates at G_0 (17). In the subsequent discussion, we refer simply to Rb rather than Rb family members, but the actions described are usually equally applicable to all three. However, when the occasion arises, we state where significant differences

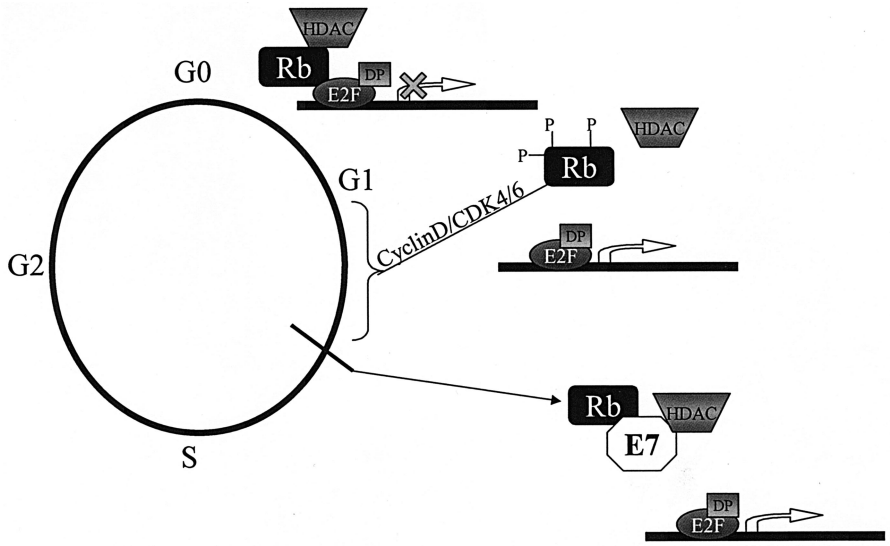

FIG. 3. Cartoon of cell cycle regulatory activities mediated by Rb, HDAC, and E2F/DP-1 proteins. The effects of E7 on Rb and HDAC binding are indicated. Rb-HDAC complexes repress the activity of E2F/DP-1 transcription complexes bound to DNA in G_1. As cells move into the S phase, Rb becomes phosphorylated by cyclin D/cdk4/6 kinases, leading to the release of Rb-HDAC proteins. E7 binds Rb and HDACs independently, resulting in the constituitive activation of E2F-inducible genes.

exist. The unphosphorylated forms of Rb form complexes with the E2F/DP1 transcription factors that are bound to the promoters of genes involved in S-phase progression or apoptosis, and this results in repression of transcription (38, 153). On progression from G_1 into the S phase, cyclin-kinase complexes phosphorylate Rb, resulting in release of Rb from E2F transcription factor complexes and transcription of genes involved in DNA synthesis (Fig. 3). The binding of E7 sequesters Rb away from E2F/DP1 complexes, resulting in the constitutive activation of the corresponding genes (38, 153). In addition to binding Rb, E7 mediates its degradation through the ubiquitin proteosome pathway (7, 38, 73, 151). Furthermore, Rb family members are major regulators of the cell cycle exit that occurs during epithelial differentiation. The abrogation of Rb function by E7 thus allows for productive replication in differentiated suprabasal cells (13). Binding of E7 to Rb has recently been shown to be important for the maintenance of episomal copies of HPV31 in undifferentiated cells (96a). Although the mechanism by which this occurs is unclear, it is probably related to abrogation of cellular checkpoints that block the maintenance of extrachromosomal DNAs.

The binding of Rb family members to E7 is not restricted to high-risk HPV types, since low-risk E7 proteins also associate with Rb, although this occurs at a much reduced affinity. Both high- and low-risk E7 proteins contain similar but not identical amino acids in the CR2 domain that mediates binding to Rb (16). A single amino acid change in the Rb binding site of low-risk viral E7 proteins can result in higher binding affinity to E7 and acquisition of the ability to transform rodent cells (59, 132). However, the E7 protein of HPV-1, a viral type associated with plantar warts, binds Rb family members with high affinity but is still not able to activate E2F-inducible genes or transform rodent cells. In addition, HPV-1 E7 is unable to degrade the Rb protein, and this may contribute to its inability to activate E2F regulated genes. This suggests that while Rb binding by E7 is very important, other factors also contribute to the role of E7 in transformation and immortalization (16, 132).

In addition to binding to Rb family members, E7 proteins associate with cyclins A and E as well as cyclin-dependent kinase (cdk) inhibitors p21 and p27 (23, 48, 75, 129, 148, 159). Since the cyclins and the associated kinases drive cell cycle progression by phosphorylating the Rb protein, it is not surprising that the E7 protein would act to enhance the activity of these proteins. High-risk E7 proteins bind cyclinA-cdk2 complexes directly, and HPV-18 E7 also binds cyclin E indirectly through p107 (104). In both cases, binding to E7 retains cdk2-associated kinase activity (104, 129). The high-risk E7 proteins also act to increase the levels of the cyclin A and E proteins, while low-risk E7 proteins have no such effect (101). Two cdk inhibitors, p27 and p21, have also been shown to bind to E7, presumably blocking their action and thus further enhancing the activities of the cdks (48, 75, 159).

The third group of proteins bound to high-risk E7 are the histone deacetylases (HDACs). The repression of E2F-inducible promoters is mediated not only through the binding of Rb but also by the action of HDACs (Fig. 3) (9, 153). In HPV-negative cells, Rb binds to HDACs and recruits them to E2F-inducible promoters. Recently, E7 proteins were shown to bind HDACs independently of their binding to Rb, and this association is important for the role of E7 in immortalization as well as episomal maintenance (96a). HDACs are expressed in all tissues and act to remove acetyl groups from the lysine-rich amino-terminal tails of the histone proteins that make up the nucleosome. In addition, HDACs can directly deacetylate E2F factors, resulting in loss of their function (100). Three classes of HDACs have been identified to date, but only the members of classes I and II have been extensively studied. The class I HDACs includes human HDACs 1, 2, 3, and 8, and these proteins are localized exclusively to the nucleus (100). Class I HDACS are active only when bound to cofactor proteins that modulate their activity or direct them to the site to be deacety-

lated. Class II HDACs include HDACs 4, 5, 6, 7, 9, and 10, and these proteins shuttle in and out of the nucleus. High-risk E7 proteins probably bind to HDACs 1 and 2 through Mi2β, which binds directly to E7 (10). The amino acids that mediate the binding of E7 to HDACS 1 and 2 include the cysteine motifs at the C terminus as well as amino acid 67. Both HPV-16 and HPV-31 E7 displace HDACs from Rb and bind these proteins independently of Rb (9).

As described above, mutation of the HDAC binding domain in HPV-31 E7 in the context of the complete viral genome results in an inability to stably maintain viral episomes as well as a failure to extend the life span of transfected cells. It is not obvious why HDAC binding to E7 would be necessary for maintenance of the viral genome, but several possibilities exist. First, since the binding of E7 to HDACs displaces them from bound Rb, this may act to unmask some important activity of Rb. A second possibility is that binding of HDACs to E7 blocks the ability to deacetylate the E2F transcription factors leading to relocalization of these factors outside the nucleus. p130 complexes, containing E2F4 and E2F5, with HDACs are the most prominent complexes found on promoters of quiescent or differentiating cells. It has been proposed that the removal of HDAC activity from the promoters allows for acetylation and subsequent promoter activation (69). In transient-transfection assays, E7 has been shown to transactivate the cdc25A phosphatase promoter through E2F binding sites in the promoter, and this activity is dependent on both the binding Rb and HDACs (113). cdc25A is important for dephosphorylation and activation of cdks and is required for cell cycle progression (74). This may be another important cellular target of E7 that is necessary for its role in viral pathogenesis. Finally, E7 silences genes via HDAC recruitment, as in the case of the interferon regulatory factor 1 (IRF-1) gene, whose expression is important for interferon signaling and immune surveillance of persisting HPV infections (118).

The C-terminal regions of both high- and low-risk E7 proteins contain conserved Cys-X-X-Cys motifs that resemble zinc finger-like domains. Since most zinc fingers are involved in binding to DNA or even RNA, this region of E7 should not strictly be referred to as such since E7 has never been shown to directly bind DNA. Mutation of one or both of the cysteines in one of the Cys-X-X-Cys motifs results in the loss of the ability to immortalize HFKs as well as to transform rodent cells (73). Recent studies have shown that the stability of the E7 protein is dramatically decreased when these cysteines are mutated, indicating an important role in maintaining the structural integrity of E7 (96a). This is consistent with evidence that the zinc finger-like motifs facilitate the binding of zinc ions and the formation of multimers of E7 (103).

One of the intriguing properties of the high-risk E7 proteins is their ability to induce genomic instability. Many HPV-positive cancers contain consistent patterns of aneuploidy, suggesting that changes in chromosome number are important events in progression (57, 138). Expression of E7 alone was shown to be sufficient to induce an increase in abnormal centrosome numbers in primary human keratinocytes (35). Centrosomes are major microtubule-organizing centers and coordinate segregation of chromosomes into daughter cells during cell division. Interestingly, E7 mutants that do not bind or degrade Rb but do associate with p107 retain the ability to induce centrosomal abnormalities (36). Centrosomal abnormalities were also seen in cells devoid of Rb and p53 as well as in Rb, p130, and p107 knockout mouse embryo fibroblasts. It is possible that the binding of a combination of Rb family members or other factors is required to mediate the appearance of centrosomal abnormalities (36). Significant advances have been made in understanding the role of high-risk E7 proteins in the development of anogenital malignancies, but more studies are required to understand the role of this protein in the productive phase of the viral life cycle.

E1 AND E2 REPLICATION PROTEINS

The most highly conserved of all papillomavirus ORFs are those encoding the E1 protein. E1 proteins are approximately 68 kDa in size and are expressed at low levels in HPV-positive cells. E1 proteins function in origin recognition and exhibit both ATPase and 3′-5′ helicase activities (65, 136, 157). They recognize AT-rich sequences at the origins of HPV replication, which are located proximal to the start sites of early transcription (44, 45, 108). By itself, E1 weakly binds origin sequences, but this binding is facilitated by complex formation with E2 proteins (31, 44, 97, 143). E2 binding sites are located adjacent to E1 recognition sequences, and E2 acts to load E1 onto the origin. Once bound, E1 proteins form hexamers that have a high affinity for DNA (135). As with other helicases, the viral DNA passes through the center of the E1 hexameric ring (95). These E1 complexes efficiently unwind supercoiled DNA with the help of chaperone proteins. E1 proteins also bind DNA polymerase α and help to recruit cellular replication complexes to the viral origin of replication (1, 18, 102). Chromatin-remodeling complexes may also be recruited by E1 so as to modulate nucleosome positioning and allow for efficient procession of the replication fork (144). The crystal structure of the DNA binding domain of E1 has identified an extended loop and α-helix that are important for recognizing DNA (39). One intriguing interaction is that of E1 with cyclins A and E, and this may act to regulate E1 activity. Four cyclin kinase phosphorylation sites are present in E1 proteins, and mutation of these sites drastically reduces the replication activity of E1 (98). It is still not clear how E1 activity is regulated in differentiating cells, but studies with HPV-31 have shown that expression of E1 transcripts shifts from the early to late promoters, resulting in increased E1 expression (29). A shift in the absolute levels of E1 or the ratios of E1 to E2 is thought to result in high-level replication upon differentiation.

The E2 protein is required for both the replication of viral DNA and transcriptional regulation (90). E2 proteins are approximately 50 kDa in size and function as dimers. The C terminus encodes a DNA binding domain that has been crystallized and shown to consist of a dimeric β-barrel structure that bends DNA (60). The N terminus contains a transactivation domain, while the C terminus interacts with E1 (14). The N terminus has also been crystallized and consists of a glutamine-rich α-helix packed against a β-sheet framework (3, 56). E2 dimers bind to consensus palindromic sequences (AC CN6GGT) called E2 binding (E2BSs) (90). There are four E2BSs present in the URR, and three of these flank the E1 recognition sequences in the origin of replication (63). On infection, early-gene transcription is activated primarily by cel-

lular transcription factors binding sequences in the URR (139). At low concentrations, E2 further activates early-gene expression, while at high concentrations it represses by interfering with the binding of transcription factors such as TFIID and Sp1 to their recognition sequences, which overlap the E2BS (28, 33). This regulation of viral expression contributes to copy number control in undifferentiated cells. On differentiation, there is a switch to the late promoter which is not repressed by E2, resulting in increased E1 and E2 expression leading to viral DNA amplification (83). E2 may also form a complex with C/EBP transcription factors, which regulate many promoters of genes involved in differentiation (55). Aside from its role in the regulation of transcription, overexpression of the E2 protein can induce apoptosis by a p53-independent mechanism (34, 52). Also, overexpression of E2 in HeLa cells was demonstrated to cause accumulation of p53 but not transcription of downstream genes like Bax. E6 overexpression in this system was unable to block the resultant apoptosis (30). Furthermore, in HeLa cells, introduction of heterologous expression vectors for E2 results in a suppression of transcription of the endogenous E6 and E7 genes, leading to senescence (51). This indicates that the continued expression of E6 and E7 is required to maintain the transformed phenotype of cervical cancer cells (51, 154).

E1^E4 AND E5 LATE PROTEINS

The HPV E4 and E5 ORFs are expressed during the early phases of the viral life cycle but only as the third and fourth ORFs on polycistronic transcripts. Since HPVs are thought to use a leaky ribosome-scanning mechanism to translate proteins from polycistronic mRNAs, little if any E4 or E5 protein is likely to be synthesized from these transcripts (125). In contrast, on epithelial-cell differentiation, E4 and E5 are expressed as the first and second ORFs of late transcripts. It is therefore likely that these proteins are synthesized primarily in the late phases of the HPV life cycle, with E4 being the most highly expressed of all HPV proteins (63). The E4 ORF is translated from spliced transcripts as a fusion with the first 5 amino acids of E1 to generate E1^E4 fusion proteins. The E4 ORF lacks an initiator AUG codon and uses the E1 sequence for translation initiation (63). All papillomaviruses express E1^E4 proteins in the late phase of the viral life cycle but have only limited sequence homology. E1^E4 proteins from the high-risk types associate with keratin networks in cells and, when overexpressed in transient-transfection assays, can induce their collapse (32). This suggests a role for E1^E4 in facilitating viral egress, but in natural infections of high-risk types only a limited amount of collapse has been observed. High-risk HPV E4 proteins may also play a role in regulating gene expression, since they interact with an RNA helicase, E4-DBD, which is a member of a helicase family involved in mRNA splicing, transport as well as initiation of translation (158). It has further been shown that overexpression of HPV-11 and HPV-16 E1^E4 induces a G_2 arrest in a variety of cell types (24). It is not clear what role E1^E4-induced cell cycle arrest plays in the productive life cycle, but it may be to counteract the effects of E7, which acts to push cells into the S phase (12, 41). Further study of the role of the E1^E4 proteins in the natural context of the complete viral genome should help to elucidate its normal function.

The HPV E5 proteins are small hydrophobic proteins whose functions remain unresolved. These proteins are localized to endosomal membranes and the Golgi but on occasion are found in the cellular membranes (19). In bovine papillomaviruses (BPVs), the E5 protein encodes the primary transforming activity and acts by associating with the platelet-derived growth factor (PDGF) receptor (133). The HPV E5 proteins have little homology to their BPV counterparts and probably act through different cellular targets. It has been suggested that HPV E5 action binds the epidermal growth factor (EGF) receptor and that it functions in a manner similar to BPV E5 and the PDGF receptor. This is based in part on the observation that overexpression of HPV E5 increases the phosphorylation of the EGF receptor as well as inhibits the degradation of the receptor (19, 127, 140, 141). Furthermore, the addition of EGF to HFKs expressing E5 alone results in increased proliferation (22). HPV E5 is likely to be expressed primarily during the late phase of the life cycle in differentiated epithelial cells. In the context of the entire HPV-31 genome, E5 was shown to only minimally influence the levels and phosphorylation status of the EGF receptor in both differentiated and undifferentiated cells (40). In addition, loss of E5 resulted in impaired activation of late viral functions in differentiating cells, suggesting that its primary activity is in differentiated cells (40).

CONCLUSIONS AND FUTURE DIRECTIONS

Our understanding of the pathogenesis of HPVs is growing rapidly. Many of the cellular targets of the high-risk E6 and E7 proteins have been identified. However, it remains important to ensure that each of these interactions represents a physiologically significant association. In contrast to the high-risk proteins, little is known about the action of the low-risk E6 and E7 proteins, since they clearly must play important roles in the viral life cycle, and this remains a useful area for study. Equally important are structural analyses of the E6 and E7 proteins either by themselves or in combination with their substrates. Such information would help to elucidate their mechanisms of action and would provide important insights that could be applied to rational drug design. The crystal structures of the viral replication proteins E1 and E2 have yielded significant insights into the action of these factors. Similarly, studies of the molecular interactions of these proteins between themselves and cellular replication proteins at origins have provided useful information about basic mechanisms of replication control. Further investigation into the other activities that these proteins mediate in viral pathogenesis, such as their roles in plasmid segregation and chromatin remodeling, is required. The function of the E4 and E5 proteins is also an area of active study and appears to center on modulating the late phase of the viral life cycle.

The differentiation-dependent HPV life cycle has been difficult to study in the past due to the lack of genetic systems and methods to differentiate epithelial cells efficiently in tissue culture. Techniques have now been developed to allow such analyses to be readily performed (47, 105). It is important that the activities of viral proteins be examined in their proper physiological contexts in complete viral genomes rather than relying

on analyses involving overexpression assays in heterologous cell types. Another major area for investigation will be the examination of the mechanisms and factors that mediate viral entry. The tools and methods are now available to address this important question, which will provide insights into viral tropism. An equally important area for investigation is the analysis of the innate and cellular immune responses to HPV infection. The lack of a good mouse model for HPV infection has hampered these studies, but these questions remain highly important and need to be addressed.

Equally important as the investigation into the basic mechanisms of HPV pathogenesis is the development of effective therapeutics to treat or prevent HPV-induced disease. A significant advance in this area has been made through the use of virus-like particle-based vaccines (85). Initial studies suggest that this vaccine will be effective in blocking initial infection, and it is anticipated that it will prevent the development of HPV-induced malignancies. While this seems likely, cervical cancers arise from single transformed cells, and it will be important to demonstrate the effectiveness of the vaccine in this process. At the same time, it is unlikely that the vaccine will benefit individuals already infected with HPV. The identification of drugs specifically to treat HPV infection has not been highly successful due to the complexities of the HPV life cycle and the limited number of enzymatic activities identified for HPV proteins. The development of drug treatments for existing HPV disease is an important undertaking that deserves further attention. In this regard, the development of therapeutic vaccines is a promising area of investigation and needs to be further supported. In summary, much has been accomplished in expanding our knowledge of this important human pathogen but, clearly, more needs to be done.

ACKNOWLEDGMENTS

M.S.L. was supported by National Cancer Institute Carcinogenesis training grant T32CA09560. L.A.L. was supported by grants from the National Institute of Allergy and Infectious Diseases and the National Cancer Institute.

REFERENCES

1. **Amin, A. A., S. Titolo, A. Pelletier, D. Fink, M. G. Cordingley, and J. Archambault.** 2000. Identification of domains of the HPV11 E1 protein required for DNA replication in vitro. Virology **272:**137–150.
2. **Androphy, E. J., N. L. Hubbert, J. T. Schiller, and D. R. Lowy.** 1987. Identification of the HPV-16 E6 protein from transformed mouse cells and human cervical carcinoma cell lines. EMBO J. **6:**989–992.
3. **Antson, A. A., J. E. Burns, O. V. Moroz, D. J. Scott, C. M. Sanders, I. B. Bronstein, G. G. Dodson, K. S. Wilson, and N. J. Maitland.** 2000. Structure of the intact transactivation domain of the human papillomavirus E2 protein. Nature **403:**805–809.
4. **Baker, C. C., W. C. Phelps, V. Lindgren, M. J. Braun, M. A. Gonda, and P. M. Howley.** 1987. Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. J. Virol. **61:**962–971.
5. **Banks, L., P. Spence, E. Androphy, N. Hubbert, G. Matlashewski, A. Murray, and L. Crawford.** 1987. Identification of human papillomavirus type 18 E6 polypeptide in cells derived from human cervical carcinomas. J. Gen. Virol. **68:**1351–1359.
6. **Bedell, M. A., K. H. Jones, S. R. Grossman, and L. A. Laimins.** 1989. Identification of human papillomavirus type 18 transforming genes in immortalized and primary cells. J. Virol. **63:**1247–1255.
7. **Berezutskaya, E., B. Yu, A. Morozov, P. Raychaudhuri, and S. Bagchi.** 1997. Differential regulation of the pocket domains of the retinoblastoma family proteins by the HPV16 E7 oncoprotein. Cell Growth Differ. **8:**1277–1286.
8. **Boring, C. C., T. S. Squires, T. Tong, and S. Montgomery.** 1994. Cancer statistics, 1994. CA Cancer J. Clin. **44:**7–26.
9. **Brehm, A., E. A. Miska, D. J. McCance, J. L. Reid, A. J. Bannister, and T. Kouzarides.** 1998. Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature **391:**597–601.
10. **Brehm, A., S. J. Nielsen, E. A. Miska, D. J. McCance, J. L. Reid, A. J. Bannister, and T. Kouzarides.** 1999. The E7 oncoprotein associates with Mi2 and histone deacetylase activity to promote cell growth. EMBO J. **18:**2449–2458.
11. **Butz, K., and F. Hoppe-Seyler.** 1993. Transcriptional control of human papillomavirus (HPV) oncogene expression: composition of the HPV type 18 upstream regulatory region. J. Virol. **67:**6476–6486.
12. **Chang, Y. E., and L. A. Laimins.** 2000. Microarray analysis identifies interferon-inducible genes and Stat-1 as major transcriptional targets of human papillomavirus type 31. J. Virol. **74:**4174–4182.
13. **Chellappan, S., V. B. Kraus, B. Kroger, K. Munger, P. M. Howley, W. C. Phelps, and J. R. Nevins.** 1992. Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between transcription factor E2F and the retinoblastoma gene product. Proc. Natl. Acad. Sci. USA **89:**4549–4553.
14. **Chen, G., and A. Stenlund.** 2000. Two patches of amino acids on the E2 DNA binding domain define the surface for interaction with E1. J. Virol. **74:**1506–1512.
15. **Cheng, S., D. Schmidt-Grimminger, T. Murant, T. Broker, and L. Chow.** 1995. Differentiation-dependent up-regulation of the human papillomavirus E7 gene reactivates cellular DNA replication in suprabasal differentiated keratinocytes. Genes Dev. **9:**2335–2349.
16. **Ciccolini, F., G. Di Pasquale, F. Carlotti, L. Crawford, and M. Tommasino.** 1994. Functional studies of E7 proteins from different HPV types. Oncogene **9:**2633–2638.
17. **Classon, M., and N. Dyson.** 2001. p107 and p130: versatile proteins with interesting pockets. Exp. Cell Res. **264:**135–147.
18. **Conger, K. L., J. S. Liu, S. R. Kuo, L. T. Chow, and T. S. Wang.** 1999. Human papillomavirus DNA replication. Interactions between the viral E1 protein and two subunits of human dna polymerase alpha/primase. J. Biol. Chem. **274:**2696–2705.
19. **Conrad, M., V. J. Bubb, and R. Schlegel.** 1993. The human papillomavirus type 6 and 16 E5 proteins are membrane-associated proteins which associate with the 16-kilodalton pore-forming protein. J. Virol. **67:**6170–6178.
20. **Craven, S. E., and D. S. Bredt.** 1998. PDZ proteins organize synaptic signaling pathways. Cell **93:**495–498.
21. **Cripe, T. P., T. H. Haugen, J. P. Turk, F. Tabatabai, P. G. Schmid III, M. Durst, L. Gissmann, A. Roman, and L. P. Turek.** 1987. Transcriptional regulation of the human papillomavirus-16 E6–E7 promoter by a keratinocyte-dependent enhancer, and by viral E2 *trans*-activator and repressor gene products: implications for cervical carcinogenesis. EMBO J. **6:**3745–3753.
22. **Crusius, K., E. Auvinen, and A. Alonso.** 1997. Enhancement of EGF- and PMA-mediated MAP kinase activation in cells expressing the human papillomavirus type 16 E5 protein. Oncogene **15:**1437–1444.
23. **Davies, R., R. Hicks, T. Crook, J. Morris, and K. Vousden.** 1993. Human papillomavirus type 16 E7 associates with a histone H1 kinase and with p107 through sequences necessary for transformation. J. Virol. **67:**2521–2528.
24. **Davy, C. E., D. J. Jackson, Q. Wang, K. Raj, P. J. Masterson, N. F. Fenner, S. Southern, S. Cuthill, J. B. Millar, and J. Doorbar.** 2002. Identification of a G_2 arrest domain in the E1 wedge E4 protein of human papillomavirus type 16. J. Virol. **76:**9806–9818.
25. **Degenhardt, Y. Y., and S. Silverstein.** 2001. Interaction of zyxin, a focal adhesion protein, with the e6 protein from human papillomavirus type 6 results in its nuclear translocation. J. Virol. **75:**11791–11802.
26. **Degenhardt, Y. Y., and S. J. Silverstein.** 2001. Gps2, a protein partner for human papillomavirus E6 proteins. J. Virol. **75:**151–160.
27. **del Mar Pena, L. M., and L. A. Laimins.** 2001. Differentiation-dependent chromatin rearrangement coincides with activation of human papillomavirus type 31 late gene expression. J. Virol. **75:**10005–10013.
28. **Demeret, C., C. Desaintes, M. Yaniv, and F. Thierry.** 1997. Different mechanisms contribute to the E2-mediated transcriptional repression of human papillomavirus type 18 viral oncogenes. J. Virol. **71:**9343–9349.
29. **Deng, W., G. Jin, B. Y. Lin, B. A. Van Tine, T. R. Broker, and L. T. Chow.** 2003. mRNA splicing regulates human papillomavirus type 11 E1 protein production and DNA replication. J. Virol. **77:**10213–10226.
30. **Desaintes, C., S. Goyat, S. Garbay, M. Yaniv, and F. Thierry.** 1999. Papillomavirus E2 induces p53-independent apoptosis in HeLa cells. Oncogene **18:**4538–4545.
31. **Dixon, E. P., G. L. Pahel, W. J. Rocque, J. A. Barnes, D. C. Lobe, M. H. Hanlon, K. A. Alexander, S. F. Chao, K. Lindley, and W. C. Phelps.** 2000. The E1 helicase of human papillomavirus type 11 binds to the origin of replication with low sequence specificity. Virology **270:**345–357.
32. **Doorbar, J., S. Ely, J. Sterling, C. McLean, and L. Crawford.** 1991. Specific interaction between HPV-16 E1–E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. Nature **352:**824–827.
33. **Dostatni, N., P. F. Lambert, R. Sousa, J. Ham, P. M. Howley, and M. Yaniv.** 1991. The functional BPV-1 E2 trans-activating protein can act as a repressor by preventing formation of the initiation complex. Genes Dev. **5:**1657–1671.
34. **Dowhanick, J. J., A. A. McBride, and P. M. Howley.** 1995. Suppression of

cellular proliferation by the papillomavirus E2 protein. J. Virol. **69:**7791–7799.

35. **Duensing, S., L. Y. Lee, A. Duensing, J. Basile, S. Piboonniyom, S. Gonzalez, C. P. Crum, and K. Munger.** 2000. The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. Proc. Natl. Acad. Sci. USA **97:**10002–10007.
36. **Duensing, S., and K. Munger.** 2003. Human papillomavirus type 16 E7 oncoprotein can induce abnormal centrosome duplication through a mechanism independent of inactivation of retinoblastoma protein family members. J. Virol. **77:**12331–12335.
37. **Dyson, N., P. M. Howley, K. Munger, and E. Harlow.** 1989. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science **243:**934–937.
38. **Edmonds, C., and K. H. Vousden.** 1989. A point mutational analysis of human papillomavirus type 16 E7 protein. J. Virol. **63:**2650–2656.
39. **Enemark, E. J., G. Chen, D. E. Vaughn, A. Stenlund, and L. Joshua-Tor.** 2000. Crystal structure of the DNA binding domain of the replication initiation protein E1 from papillomavirus. Mol. Cell **6:**149–158.
40. **Fehrmann, F., D. J. Klumpp, and L. A. Laimins.** 2003. Human papillomavirus type 31 E5 protein supports cell cycle progression and activates late viral functions upon epithelial differentiation. J. Virol. **77:**2819–2831.
41. **Flores, E., B. L. Allen-Hoffman, D. Lee, and P. F. Lambert.** 2000. The human papillomavirus type 16 E7 oncogene is required for the productive stage of the viral life cycle. J. Virol. **74:**6622–6631.
42. **Foster, S. A., G. W. Demers, B. G. Etscheid, and D. A. Galloway.** 1994. The ability of human papillomavirus E6 proteins to target p53 for degradation in vivo correlates with their ability to abrogate actinomycin D-induced growth arrest. J. Virol. **68:**5698–5705.
43. **Frame, M. C.** 2002. Src in cancer: deregulation and consequences for cell behaviour. Biochim. Biophys. Acta **1602:**114–130.
44. **Frattini, M. G., and L. A. Laimins.** 1994. Binding of the human papillomavirus E1 origin-recognition protein is regulated through complex formation with the E2 enhancer-binding protein. Proc. Natl. Acad. Sci. USA **91:**12398–12402.
45. **Frattini, M. G., and L. A. Laimins.** 1994. The role of the E1 and E2 proteins in the replication of human papillomavirus type 31b. Virology **204:**799–804.
46. **Frattini, M. G., H. B. Lim, J. Doorbar, and L. A. Laimins.** 1997. Induction of human papillomavirus type 18 late gene expression and genomic amplification in organotypic cultures from transfected DNA templates. J. Virol. **71:**7068–7072.
47. **Frattini, M. G., H. B. Lim, and L. A. Laimins.** 1996. In vitro synthesis of oncogenic human papillomaviruses requires episomal genomes for differentiation-dependent late expression. Proc. Natl. Acad. Sci. USA **93:**3062–3067.
48. **Funk, J. O., S. Waga, J. B. Harry, E. Espling, B. Stillman, and D. A. Galloway.** 1997. Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV-16 E7 oncoprotein. Genes Dev. **11:**2090–2100.
49. **Gloss, B., H. U. Bernard, K. Seedorf, and G. Klock.** 1987. The upstream regulatory region of the human papilloma virus-16 contains an E2 protein-independent enhancer which is specific for cervical carcinoma cells and regulated by glucocorticoid hormones. EMBO J. **6:**3735–3743.
50. **Gomperts, S. N.** 1996. Clustering membrane proteins: It's all coming together with the PSD-95/SAP90 protein family. Cell **84:**659–662.
51. **Goodwin, E. C., and D. DiMaio.** 2000. Repression of human papillomavirus oncogenes in HeLa cervical carcinoma cells causes the orderly reactivation of dormant tumor suppressor pathways. Proc. Natl. Acad. Sci. USA **97:**12513–12518.
52. **Goodwin, E. C., E. Yang, C. J. Lee, H. W. Lee, D. DiMaio, and E. S. Hwang.** 2000. Rapid induction of senescence in human cervical carcinoma cells. Proc. Natl. Acad. Sci. USA **97:**10978–10983.
53. **Grassman, K., B. Rapp, H. Maschek, K. U. Petry, and T. Iftner.** 1996. Identification of a differentiation-inducible promoter in the E7 open reading frame of human papillomavirus type 16 (HPV-16) in raft cultures of a new cell line containing high copy numbers of episomal HPV-16 DNA. J. Virol. **70:**2339–2349.
54. **Greenberg, R. A., R. C. O'Hagan, H. Deng, Q. Xiao, S. R. Hann, R. R. Adams, S. Lichtsteiner, L. Chin, G. B. Morin, and R. A. DePinho.** 1999. Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. Oncogene **18:**1219–1226.
55. **Hadaschik, D., K. Hinterkeuser, M. Oldak, H. J. Pfister, and S. Smola-Hess.** 2003. The papillomavirus E2 protein binds to and synergizes with C/EBP factors involved in keratinocyte differentiation. J. Virol. **77:**5253–5265.
56. **Harris, S. F., and M. R. Botchan.** 1999. Crystal structure of the human papillomavirus type 18 E2 activation domain. Science **284:**1673–1677.
57. **Hashida, T., and S. Yasumoto.** 1991. Induction of chromosome abnormalities in mouse and human epidermal keratinocytes by the human papillomavirus type 16 E7 oncogene. J. Gen. Virol. **72:**1569–1577.
58. **Hawley-Nelson, P., K. H. Vousden, N. L. Hubbert, D. R. Lowy, and J. T. Schiller.** 1989. HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. EMBO J. **8:**3905–3910.
59. **Heck, D. V., C. L. Yee, P. M. Howley, and K. Munger.** 1992. Efficiency of binding the retinoblastoma protein correlates with the transforming capacity of the E7 oncoproteins of the human papillomaviruses. Proc. Natl. Acad. Sci. USA **89:**4442–4446.
60. **Hegde, R. S., S. R. Grossman, L. A. Laimins, and P. B. Sigler.** 1992. Crystal structure at 1.7 A of the bovine papillomavirus-1 E2 DNA-binding domain bound to its DNA target. Nature **359:**505–512.
61. **Herrero, R., X. Castellsague, M. Pawlita, J. Lissowska, F. Kee, P. Balaram, T. Rajkumar, H. Sridhar, B. Rose, J. Pintos, L. Fernandez, A. Idris, M. J. Sanchez, A. Nieto, R. Talamini, A. Tavani, F. X. Bosch, U. Reidel, P. J. Snijders, C. J. Meijer, R. Viscidi, N. Munoz, and S. Franceschi.** 2003. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J. Natl. Cancer Inst. **95:**1772–1783.
62. **Hopfl, R., K. Heim, N. Christensen, K. Zumbach, U. Wieland, B. Volgger, A. Widschwendter, S. Haimbuchner, E. Muller-Holzner, M. Pawlita, H. Pfister, and P. Fritsch.** 2000. Spontaneous regression of CIN and delayed-type hypersensitivity to HPV-16 oncoprotein E7. Lancet **356:**1985–1986.
63. **Howley, P. M.** 1996. Papillomaviridae: the viruses and their replication, p. 947–978. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology, 3rd ed. Lippincott-Raven Publishers, Philadelphia, Pa.
64. **Hubbert, N. L., S. A. Sedman, and J. T. Schiller.** 1992. Human papillomavirus type 16 E6 increases the degradation rate of p53 in human keratinocytes. J. Virol. **66:**6237–6241.
65. **Hughes, F. J., and M. A. Romanos.** 1993. E1 protein of human papillomavirus is a DNA helicase/ATPase. Nucleic Acids Res. **21:**5817–5823.
66. **Huibregtse, J. M., M. Scheffner, and P. M. Howley.** 1991. A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. EMBO J. **10:**4129–4135.
67. **Huibregtse, J. M., M. Scheffner, and P. M. Howley.** 1993. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. Mol. Cell. Biol. **13:**775–784.
68. **Hummel, M., J. B. Hudson, and L. A. Laimins.** 1992. Differentiation-induced and constitutive transcription of human papillomavirus type 31b in cell lines containing viral episomes. J. Virol. **66:**6070–6080.
69. **Hurford, R. K., Jr., D. Cobrinik, M. H. Lee, and N. Dyson.** 1997. pRB and p107/p130 are required for the regulated expression of different sets of E2F responsive genes. Genes Dev. **11:**1447–1463.
70. **Ishiji, T., M. J. Lace, S. Parkkinen, R. D. Anderson, T. H. Haugen, T. P. Cripe, J. H. Xiao, I. Davidson, P. Chambon, and L. P. Turek.** 1992. Transcriptional enhancer factor (TEF)-1 and its cell-specific co-activator activate human papillomavirus-16 E6 and E7 oncogene transcription in keratinocytes and cervical carcinoma cells. EMBO J. **11:**2271–2281.
71. **Jenson, A. B., R.J. Kurman, and W. D. Lancaster.** 1991. Tissue effects of and host response to human papillomavirus infection. Dermatol. Clin. **9:**203–209.
72. **Jeon, S., B. L. Allen-Hoffmann, and P. F. Lambert.** 1995. Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. J. Virol. **69:**2989–2997.
73. **Jewers, R. J., P. Hildebrandt, J. W. Ludlow, B. Kell, and D. J. McCance.** 1992. Regions of human papillomavirus type 16 E7 oncoprotein required for immortalization of human keratinocytes. J. Virol. **66:**1329–1335.
74. **Jinno, S., K. Suto, A. Nagata, M. Igarashi, Y. Kanaoka, H. Nojima, and H. Okayama.** 1994. Cdc25A is a novel phosphatase functioning early in the cell cycle. EMBO J. **13:**1549–1556.
75. **Jones, D. L., R. M. Alani, and K. Munger.** 1997. The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21Cip1-mediated inhibition of cdk2. Genes Dev. **11:**2101–2111.
76. **Joyce, J. G., J. S. Tung, C. T. Przysiecki, J. C. Cook, E. D. Lehman, J. A. Sands, K. U. Jansen, and P. M. Keller.** 1999. The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. J. Biol. Chem. **274:**5810–5822.
77. **Kao, W. H., S. L. Beaudenon, A. L. Talis, J. M. Huibregtse, and P. M. Howley.** 2000. Human papillomavirus type 16 E6 induces self-ubiquitination of the E6AP ubiquitin-protein ligase. J. Virol. **74:**6408–6417.
78. **Kessis, T. D., R. J. Slebos, W. G. Nelson, M. B. Kastan, B. S. Plunkett, S. M. Han, A. T. Lorincz, L. Hedrick, and K. R. Cho.** 1993. Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage. Proc. Natl. Acad. Sci. USA **90:**3988–3992.
79. **Kiyono, T., S. A. Foster, J. I. Koop, J. K. McDougall, D. A. Galloway, and A. J. Klingelhutz.** 1998. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature **396:**84–88.
80. **Kiyono, T., A. Hiraiwa, M. Fujita, Y. Hayashi, T. Akiyama, and M. Ishibashi.** 1997. Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the *Drosophila* discs large tumor suppressor protein. Proc. Natl. Acad. Sci. USA **94:**11612–11616.
81. **Kjellberg, L., G. Hallmans, A. M. Ahren, R. Johansson, F. Bergman, G.**

Wadell, T. Angstrom, and J. Dillner. 2000. Smoking, diet, pregnancy and oral contraceptive use as risk factors for cervical intra-epithelial neoplasia in relation to human papillomavirus infection. Br. J. Cancer **82:**1332–1338.
82. **Klingelhutz, A. J., S. A. Foster, and J. K. McDougall.** 1996. Telomerase activation by the E6 gene product of human papillomavirus type 16. Nature **380:**79–82.
83. **Klumpp, D. J., and L. A. Laimins.** 1999. Differentiation-induced changes in promoter usage for transcripts encoding the human papillomavirus type 31 replication protein E1. Virology **257:**239–246.
84. **Ko, L., and C. Prives.** 1996. p53: puzzle and paradigm. Genes Dev. **10:** 1054–1072.
85. **Koutsky, L. A., K. A. Ault, C. M. Wheeler, D. R. Brown, E. Barr, F. B. Alvarez, L. M. Chiacchierini, and K. U. Jansen.** 2002. A controlled trial of a human papillomavirus type 16 vaccine. N. Engl. J. Med. **347:**1645–1651.
86. **Kuhne, C., and L. Banks.** 1998. E3-ubiquitin ligase/E6-AP links multicopy maintenance protein 7 to the ubiquitination pathway by a novel motif, the L2G box. J. Biol. Chem. **273:**34302–34302.
87. **Kukimoto, I., S. Aihara, K. Yoshiike, and T. Kanda.** 1998. Human papillomavirus oncoprotein E6 binds to the C-terminal region of human minichromosome maintenance 7 protein. Biochem. Biophys. Res. Commun. **249:**258–262.
88. **Kyo, S., D. J. Klumpp, M. Inoue, T. Kanaya, and L. A. Laimins.** 1997. Expression of AP1 during cellular differentiation determines human papillomavirus E6/E7 expression in stratified epithelial cells. J. Gen. Virol. **78:**401–411.
89. **Kyo, S., M. Takakura, T. Taira, T. Kanaya, H. Itoh, M. Yutsudo, H. Ariga, and M. Inoue.** 2000. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). Nucleic Acids Res. **28:**669–677.
90. **Laimins, L. A.** 1998. Regulation of transcription and replication by human papillomaviruses, p. 201–223. *In* D. J. McCance (ed.), Human tumor viruses. American Society for Microbiology, Washington, D.C.
91. **Lechner, M. S., and L. A. Laimins.** 1994. Inhibition of p53 DNA binding by human papillomavirus E6 proteins. J. Virol. **68:**4262–4273.
92. **Lee, S. S., B. Glaunsinger, F. Mantovani, L. Banks, and R. T. Javier.** 2000. Multi-PDZ domain protein MUPP1 is a cellular target for both adenovirus E4- ORF1 and high-risk papillomavirus type 18 E6 oncoproteins. J. Virol. **74:**9680–9693.
93. **Lee, S. S., R. S. Weiss, and R. T. Javier.** 1997. Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the *Drosophila* discs large tumor suppressor protein. Proc. Natl. Acad. Sci. USA **94:**6670–6675.
94. **Liu, J. P.** 1999. Studies of the molecular mechanisms in the regulation of telomerase activity. FASEB J. **13:**2091–2104.
95. **Liu, J. S., S. R. Kuo, A. M. Makhov, D. M. Cyr, J. D. Griffith, T. R. Broker, and L. T. Chow.** 1998. Human Hsp70 and Hsp40 chaperone proteins facilitate human papillomavirus-11 E1 protein binding to the origin and stimulate cell-free DNA replication. J. Biol. Chem. **273:**30704–30712.
96. **Liu, Y., J. J. Chen, Q. Gao, S. Dalal, Y. Hong, C. P. Mansur, V. Band, and E. J. Androphy.** 1999. Multiple functions of human papillomavirus type 16 E6 contribute to the immortalization of mammary epithelial cells. J. Virol. **73:**7297–7307.
96a. **Longworth, M. S., and L. A. Laimins.** 2004. The binding of histone deacetylases and the integrity of zinc finger-like motifs of the E7 protein are essential for the life cycle of human papillomavirus type 31. J. Virol. **78:** 3533–3541.
97. **Lu, J. Z., Y. N. Sun, R. C. Rose, W. Bonnez, and D. J. McCance.** 1993. Two E2 binding sites (E2BS) alone or one E2BS plus an A/T-rich region are minimal requirements for the replication of the human papillomavirus type 11 origin. J. Virol. **67:**7131–7139.
98. **Ma, T., N. Zou, B. Y. Lin, L. T. Chow, and J. W. Harper.** 1999. Interaction between cyclin-dependent kinases and human papillomavirus replication-initiation protein E1 is required for efficient viral replication. Proc. Natl. Acad. Sci. USA **96:**382–387.
99. **Marin, M., C. Jost, M. Irwin, J. DeCaprio, D. Caput, and W. Kaelin.** 1998. Viral oncoproteins discriminate between p53 and the p53 homolog p73. Mol. Cell. Biol. **18:**6316–6324.
100. **Marks, P., R. A. Rifkind, V. M. Richon, R. Breslow, T. Miller, and W. K. Kelly.** 2001. Histone deacetylases and cancer: causes and therapies. Nat. Rev. Cancer **1:**194–202.
101. **Martin, L. G., G. W. Demers, and D. A. Galloway.** 1998. Disruption of the G_1/S transition in human papillomavirus type 16 E7-expressing human cells is associated with altered regulation of cyclin E. J. Virol. **72:**975–985.
102. **Masterson, P. J., M. A. Stanley, A. P. Lewis, and M. A. Romanos.** 1998. A C-terminal helicase domain of the human papillomavirus E1 protein binds E2 and the DNA polymerase alpha-primase p68 subunit. J. Virol. **72:**7407–7419.
103. **McIntyre, M. C., M. G. Frattini, S. R. Grossman, and L. A. Laimins.** 1993. Human papillomavirus type 18 E7 protein requires intact Cys-X-X-Cys motifs for zinc binding, dimerization, and transformation but not for Rb binding. J. Virol. **67:**3142–3150.
104. **McIntyre, M. C., M. N. Ruesch, and L. A. Laimins.** 1996. Human papillomavirus E7 oncoproteins bind a single form of cyclin E in a complex with cdk2 and p107. Virology **215:**73–82.
105. **Meyers, C., M. Frattini, J. Hudson, and L. Laimins.** 1992. Biosynthesis of human papillomavirus from a continuous cell line upon epithelial differentiation. Science **257:**971–973.
106. **Meyerson, M., C. M. Counter, E. N. Eaton, L. W. Ellisen, P. Steiner, S. D. Caddle, L. Ziaugra, R. L. Beijersbergen, M. J. Davidoff, Q. Liu, S. Bacchetti, D. A. Haber, and R. A. Weinberg.** 1997. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell **90:**785–795.
107. **Mohr, I. J., R. Clark, S. Sun, E. J. Androphy, P. MacPherson, and M. R. Botchan.** 1990. Targeting the E1 replication protein to the papillomavirus origin of replication by complex formation with the E2 transactivator. Science **250:**1694–1699.
108. **Muller, F., T. Giroglou, and M. Sapp.** 1997. Characterization of the DNA-binding activity of the E1 and E2 proteins and the E1/E2 complex of human papillomavirus type 33. J. Gen. Virol. **78:**911–915.
109. **Munger, K., W. C. Phelps, V. Bubb, P. M. Howley, and R. Schlegel.** 1989. The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. J. Virol. **63:**4417–4421.
110. **Munger, K., B. Werness, N. Dyson, W. Phelps, E. Harlow, and P. Howley.** 1989. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. EMBO J. **8:**4099–4105.
111. **Nakagawa, S., and J. M. Huibregtse.** 2000. Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase. Mol. Cell. Biol. **20:**8244–8253.
112. **Nakamura, T. M., G. B. Morin, K. B. Chapman, S. L. Weinrich, W. H. Andrews, J. Lingner, C. B. Harley, and T. R. Cech.** 1997. Telomerase catalytic subunit homologs from fission yeast and human. Science **277:** 955–959.
113. **Nguyen, D. X., T. F. Westbrook, and D. J. McCance.** 2002. Human papillomavirus type 16 E7 maintains elevated levels of the cdc25A tyrosine phosphatase during deregulation of cell cycle arrest. J. Virol. **76:**619–632.
114. **Nguyen, M. L., M. M. Nguyen, D. Lee, A. E. Griep, and P. F. Lambert.** 2003. The PDZ ligand domain of the human papillomavirus type 16 E6 protein is required for E6's induction of epithelial hyperplasia in vivo. J. Virol. **77:** 6957–6964.
115. **O'Conner, M., and H.-U. Bernard.** 1995. Oct-1 activates the epithelial-specific enhancer of HPV 16 through synergistic interaction with NF-1 at a conserved regulatory site. Virology **207:**77–88.
116. **Oh, S. T., S. Kyo, and L. A. Laimins.** 2001. Telomerase activation by human papillomavirus type 16 E6 protein: induction of human telomerase reverse transcriptase expression through Myc and GC-rich Sp1 binding sites. J. Virol. **75:**5559–5566.
116a. **Oh, S. T., M. S. Longworth, and L. A. Laimins.** 2004. Roles of the E6 and E7 proteins in the life cycle of low-risk human papillomavirus type 11. J. Virol. **78:**2620–2626.
117. **Ozbun, M. A., and C. Meyers.** 1998. Temporal usage of multiple promoters during the life cycle of human papillomavirus 31b. J. Virol. **72:**2715–2722.
118. **Park, J. S., E. J. Kim, H. J. Kwon, E. S. Hwang, S. E. Namkoong, and S. J. Um.** 2000. Inactivation of interferon regulatory factor-1 tumor suppressor protein by HPV E7 oncoprotein. Implication for the E7-mediated immune evasion mechanism in cervical carcinogenesis. J. Biol. Chem. **275:**6764–6769.
119. **Park, J.-S. E-J. Kim, J-Y. Lee, H-S. Sin, S. Namkoong, and S-J. Um.** 2001. Functional inactivation of p73, a homolog of the p53 tumor suppressor protein, by HPV E6 proteins. Int. J. Cancer **91:**822–827.
120. **Parkin, D. M., F. Bray, J. Ferlay, and P. Pisani.** 2001. Estimating the world cancer burden: Globocan 2000. Int. J. Cancer **94:**153–156.
121. **Patel, D., S. Huang, L. Baglia, and D. McCance.** 1999. The E6 protein of HPV 16 binds to and inhibits co-activation by CBP and p300. EMBO J. **18:**5061–5072.
122. **Phelps, W. C., C. L. Yee, K. Munger, and P. M. Howley.** 1988. The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of adenovirus E1A. Cell **53:**539–547.
123. **Pim, D., A. Storey, M. Thomas, P. Massimi, and L. Banks.** 1994. Mutational analysis of HPV-18 E6 identifies domains required for p53 degradation in vitro, abolition of p53 transactivation in vivo and immortalisation of primary BMK cells. Oncogene **9:**1869–1876.
124. **Pisani, P., F. Bray, and D. M. Parkin.** 2002. Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. Int. J. Cancer **97:**72–81.
125. **Remm, M., A. Remm, and M. Ustav.** 1999. Human papillomavirus type 18 E1 protein is translated from polycistronic mRNA by a discontinuous scanning mechanism. J. Virol. **73:**3062–3070.
126. **Riley, R. R., S. Duensing, T. Brake, K. Munger, P. F. Lambert, and J. M. Arbeit.** 2003. Dissection of human papillomavirus E6 and E7 function in transgenic mouse models of cervical carcinogenesis. Cancer Res. **63:**4862–4871.
127. **Rodriguez, M. I., M. E. Finbow, and A. Alonso.** 2000. Binding of human

papillomavirus 16 E5 to the 16 kDa subunit c (proteolipid) of the vacuolar H^+-ATPase can be dissociated from the E5-mediated epidermal growth factor receptor overactivation. Oncogene **19:**3727–3732.
128. **Ronco, L. V., A. Y. Karpova, M. Vidal, and P. M. Howley.** 1998. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev. **12:**2061–2072.
129. **Ruesch, M. N., and L. A. Laimins.** 1998. Human papillomavirus oncoproteins alter differentiation-dependent cell cycle exit on suspension in semisolid medium. Virology **250:**19–29.
130. **Scheffner, M., J. M. Huibregtse, and P. M. Howley.** 1994. Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of p53. Proc. Natl. Acad. Sci. USA **91:**8797–8801.
131. **Scheffner, M., B. A. Werness, J. M. Huibregtse, A. J. Levine, and P. M. Howley.** 1990. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell **63:**1129–1136.
132. **Schmitt, A., J. B. Harry, B. Rapp, F. O. Wettstein, and T. Iftner.** 1994. Comparison of the properties of the E6 and E7 genes of low- and high-risk cutaneous papillomaviruses reveals strongly transforming and high Rb-binding activity for the E7 protein of the low-risk human papillomavirus type 1. J. Virol. **68:**7051–7059.
133. **Schneider-Gadicke, A., and E. Schwarz.** 1986. Different human cervical carcinoma cell lines show similar transcription patterns of human papillomavirus type 18 early genes. EMBO J. **5:**2285–2292.
134. **Schwarz, E., U. K. Freese, L. Gissmann, W. Mayer, B. Roggenbuck, A. Stremlau, and H. zur Hausen.** 1985. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature **314:**111–114.
135. **Sedman, J., and A. Stenlund.** 1998. The papillomavirus E1 protein forms a DNA-dependent hexameric complex with ATPase and DNA helicase activities. J. Virol. **72:**6893–6897.
136. **Seo, Y. S., F. Muller, M. Lusky, and J. Hurwitz.** 1993. Bovine papilloma virus (BPV)-encoded E1 protein contains multiple activities required for BPV DNA replication. Proc. Natl. Acad. Sci. USA **90:**702–706.
137. **Singer, A., L. Ho, G. Terry, and T. S. Kwie.** 1995. Association of human papillomavirus with cervical cancer and precancer, p. 105–129. *In* A. Mindel (ed.), Genital warts: human papillomavirus infection. Edward Arnold, London, United Kingdom.
138. **Solinas-Toldo, S., M. Durst, and P. Lichter.** 1997. Specific chromosomal imbalances in human papillomavirus-transfected cells during progression toward immortality. Proc. Natl. Acad. Sci. USA **94:**3854–3859.
139. **Steger, G., and S. Corbach.** 1997. Dose-dependent regulation ofte early promoter of HPV 18 by the viral E2 protein. J. Virol. **71:**50–58.
140. **Straight, S. W., B. Herman, and D. J. McCance.** 1995. The E5 oncoprotein of human papillomavirus type 16 inhibits the acidification of endosomes in human keratinocytes. J. Virol. **69:**3185–3192.
141. **Straight, S. W., P. M. Hinkle, R. J. Jewers, and D. J. McCance.** 1993. The E5 oncoprotein of human papillomavirus type 16 transforms fibroblasts and effects the downregulation of the epidermal growth factor receptor in keratinocytes. J. Virol. **67:**4521–4532.
142. **Stubenrauch, F., H. B. Lim, and L. A. Laimins.** 1998. Differential requirements for conserved E2 binding sites in the life cycle of oncogenic human papillomavirus type 31. J. Virol. **72:**1071–1077.
143. **Sun, Y. N., J. Z. Lu, and D. J. McCance.** 1996. Mapping of HPV-11 E1 binding site and determination of other important cis elements for replication of the origin. Virology **216:**219–222.
144. **Swindle, C. S., and J. A. Engler.** 1998. Association of the human papillomavirus type 11 E1 protein with histone H1. J. Virol. **72:**1994–2001.
145. **Thomas, J. T., W. G. Hubert, M. N. Ruesch, and L. A. Laimins.** 1999. Human papillomavirus type 31 oncoproteins E6 and E7 are required for the maintenance of episomes during the viral life cycle in normal human keratinocytes. Proc. Natl. Acad. Sci. USA **96:**8449–8454.
146. **Thomas, M., and L. Banks.** 1999. Human papillomavirus (HPV) E6 interactions with Bak are conserved amongst E6 proteins from high and low risk HPV types. J. Gen. Virol. **80:**1513–1517.
147. **Thompson, D. A., G. Belinsky, T. H. Chang, D. L. Jones, R. Schlegel, and K. Munger.** 1997. The human papillomavirus-16 E6 oncoprotein decreases the vigilance of mitotic checkpoints. Oncogene **15:**3025–3035.
148. **Tommasino, M., J. P. Adamczewski, F. Carlotti, C. F. Barth, R. Manetti, M. Contorni, F. Cavalieri, T. Hunt, and L. Crawford.** 1993. HPV16 E7 protein associates with the protein kinase p33CDK2 and cyclin A. Oncogene **8:**195–202.
149. **Veldman, T., X. Liu, H. Yuan, and R. Schlegel.** 2003. Human papillomavirus E6 and Myc proteins associate in vivo and bind to and cooperatively activate the telomerase reverse transcriptase promoter. Proc. Natl. Acad. Sci. USA **100:**8211–8216.
150. **Walboomers, J. M., M. V. Jacobs, M. M. Manos, F. X. Bosch, J. A. Kummer, K. V. Shah, P. J. Snijders, J. Peto, C. J. Meijer, and N. Munoz.** 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J. Pathol. **189:**12–19.
151. **Wang, J., A. Sampath, P. Raychaudhuri, and S. Bagchi.** 2001. Both Rb and E7 are regulated by the ubiquitin proteasome pathway in HPV-containing cervical tumor cells. Oncogene **20:**4740–4749.
152. **Wang, J., L. Y. Xie, S. Allan, D. Beach, and G. J. Hannon.** 1998. Myc activates telomerase. Genes Dev. **12:**1769–1774.
153. **Weintraub, S. J., K. N. Chow, R. X. Luo, S. H. Zhang, S. He, and D. C. Dean.** 1995. Mechanism of active transcriptional repression by the retinoblastoma protein. Nature **375:** 812–815.
154. **Wells, S. I., D. A. Francis, A. Y. Karpova, J. J. Dowhanick, J. D. Benson, and P. M. Howley.** 2000. Papillomavirus E2 induces senescence in HPV-positive cells via pRB- and p21(CIP)-dependent pathways. EMBO J. **19:** 5762–5771.
155. **Werness, B. A., A. J. Levine, and P. M. Howley.** 1990. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science **248:**76–79.
156. **Wu, K. J., C. Grandori, M. Amacker, N. Simon-Vermot, A. Polack, J. Lingner, and R. Dalla-Favera.** 1999. Direct activation of TERT transcription by c-MYC. Nat. Genet. **21:**220–224.
157. **Yang, L., I. Mohr, E. Fouts, D. A. Lim, M. Nohaile, and M. Botchan.** 1993. The E1 protein of bovine papilloma virus 1 is an ATP-dependent DNA helicase. Proc. Natl. Acad. Sci. USA **90:**5086–5090.
158. **Yoshioka, N., H. Inoue, K. Nakanishi, K. Oka, M. Yutsudo, A. Yamashita, A. Hakura, and H. Nojima.** 2000. Isolation of transformation suppressor genes by cDNA subtraction: lumican suppresses transformation induced by v-src and v-K- ras. J. Virol. **74:**1008–1013.
159. **Zerfass-Thome, K., W. Zwerschke, B. Mannhardt, R. Tindle, J. W. Botz, and P. Jansen-Durr.** 1996. Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. Oncogene **13:**2323–2330.
160. **Zimmermann, H., R. Degenkolbe, H. U. Bernard, and M. J. O'Connor.** 1999. The human papillomavirus type 16 E6 oncoprotein can down-regulate p53 activity by targeting the transcriptional coactivator CBP/p300. J. Virol. **73:**6209–6219.
161. **zur Hausen, H.** 1996. Papillomavirus infections—a major cause of human cancers. Biochim. Biophys. Acta **1288:**F55–F78.
162. **zur Hausen, H.** 2002. Papillomaviruses and cancer: from basic studies to clinical application. Nat. Rev. Cancer **2:**342–350.